EXHIBIT C

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Characteristic features of Japanese women's hair with aging and with progressing hair loss

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Summary

Background: There have been few studies of the features of hair with aging and hair loss in Japanese women.
Objective: Features of Japanese women's hair with aging and with progressing hair loss were investigated.
Methods: Japanese women with hair loss ($n = 46$) or with no or less hair loss ($n = 113$), aged 14–68 years, were studied. Severity of hair loss was rated by visual comparison with six standard photographs. Hair density, hair growth rate, and hair diameter were analyzed by phototrichogram. Follicular units were deduced by a non-invasive method using tree-view analysis on scalp imaging.
Results: Hair loss in Japanese women is commonly characterized by a diffuse central pattern occurring after approximately 40 years of age. Hair density declines with age after the 40s. The reduction resulted from an increase in the number of one-haired follicular units and a reduction of three- and more-haired follicular units. Both the ratio and the growth rate of anagen hair also declined with age after the 40s. Mean hair diameter and the ratio of thick hairs increased with age from about 10 to 40 years, and decreased with progressing hair loss. There were few vellus-like hairs in women with hair loss, in comparison with male-pattern baldness.
Conclusion: In Japanese middle-aged women, hair density declined with age without the appearance of hair loss. Hair loss appeared after approximately 40 years of age. The major causes might be reduction of hair density and the ratio of thick hairs, but not an increase of vellus-like hairs.

Abbreviation: FPHL, female-pattern hair loss

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1. Introduction

Ludwig indicated that the clinical features of 'androgenetic alopecia' in females differed from common baldness in men [1], and that it presents with diffuse thinning over the top of the scalp and an intact frontal hairline, but total hair loss is rare. The role of androgens in women with hair loss remains unclear. Recently, it was proposed that 'female-pattern hair loss' (FPHL) is a condition characterized by a reduction in hair density in the central scalp, and histologically by miniaturization of some follicles and an increased percentage of hair in telogen in the thinning area [2]. Several investigators have failed to find evidence of raised androgen levels in women with hair loss, and in all studies there is a variable proportion of women with hair loss who do not show clinical or biochemical signs of androgen excess [3,4]. The alternative explanation is that non-androgenic mechanisms are involved in mediating hair loss in some women with FPHL [4]. Therefore, FPHL is now the preferred term for androgenetic alopecia in females [5], but remains a poorly understood disorder despite its frequency in the general population.

FPHL is a clinical phenotype that is likely to have various etiologies, all of which are potentially overlapping. Although classified as a non-scarring or reversible disorder, it can lead to permanent hair loss. It can be difficult to diagnose, because female hair loss also occurs with aging. It was reported that, in 377 Caucasian women aged 18–99 years, the prevalence of FPHL increased in subjects aged 60 years and over, and that low hair density was the major factor responsible for the clinical diagnosis of FPHL [6]. Furthermore, mean hair density on the frontal scalp became progressively lower with increasing age after about 40 years in women in the UK, with or without hair loss [6,7].

Racial differences in hair physiology and properties such as color, diameter, shape of cross section, twist characteristics, and the prevalence of hair thinning are known. The prevalence of hair loss in Korean women was lower than that in Caucasians [8]. In Japanease women, severe hair loss such as Ludwig grade III is rare, and hair density was a more important parameter of hair loss than hair diameter, hair growth rate, and the ratio of short hairs in a cluster analysis of 101 subjects [9]. The most prevalent pattern in Japanese women is a decrease in hair density without vellus hair changes [9].

The purpose of this study was to more precisely determine the pattern of hair loss in Japanese women. We investigated the characteristic features of hair with progressing hair loss and with age in Japanese women, and compared them with FPHL in other countries.

2. Subjects and methods

2.1. Subjects

Forty-six Japanese female volunteers with hair loss and 113 Japanese female volunteers with no or little hair loss participated. Their hair color was mainly almost black; in some cases, it was partially gray (below 10% of total hair). All subjects were healthy and free of medication. Written informed consent was obtained before the studies.

2.2. Evaluation of hair loss

Scalp hairs of the subjects were cleaned, dried, and combed along a coronal midline before photography. Photographs of the scalp top view with a light blue background were taken at fixed focus (50 mm) and at distance (1 m) using a camera (Olympus OM-4Ti, Olympus Optical Co. Ltd., Tokyo, Japan) with a film (Fujicolor Superia 100, Fuji Photo Film Co. Ltd., Tokyo, Japan), a lens (Zoom-Nikkor 28–105 mm *f*/3.5–4.5 D (IF), Nikon Corporation Tokyo, Japan), a flash (Olympus T-10 Ring Flash 1, Olympus Optical Co. Ltd.), and a polarized filter (Olympus Ring Cross Filter Pol., Olympus Optical Co. Ltd.). Four investigators visually rated each photograph in terms of stage number, or number and a half, compared with standard photographs of hair loss severity, on a 6-point scale from stage 1 (no hair loss) to stage 6 (detectable hair loss) (Fig. 1). The mean rating was taken as the severity of hair loss.

2.3. Phototrichogram

Hairs in a quadrangle of approximately 5 mm × 5 mm, located 40 mm from the coronal midline on the scalp to the ear, were cut short using ophthalmologic scissors [10]. The cut area was digitally imaged using a video microscope (VH-6300, Keyence Corporation, Osaka, Japan) with a 30-fold magnification lens, at the time of clipping (Day 0) and 2 days after (Day 2). The cut area was quantified in pixel numbers using image processing software (Mac Scope ver. 2.56, Mitani Corp., Maruoka, Fukui, Japan). Hair numbers in the area were counted, and hair density was calculated as the number of hairs per square centimeter. The lengths of the same hair on Day 0 and Day 2 were measured on the image, and growth length was obtained by their subtraction. Hair diameter was measured on the image relative to a 10 μm mark between 20 μm and 140 μm. The following parameters were then calculated: hair density (number of hairs per cm^2); hair growth rate (μm per day); ratio of anagen hair (%); average hair diameter (μm); ratio of thick hair (%); and ratio of thin hair (%). An

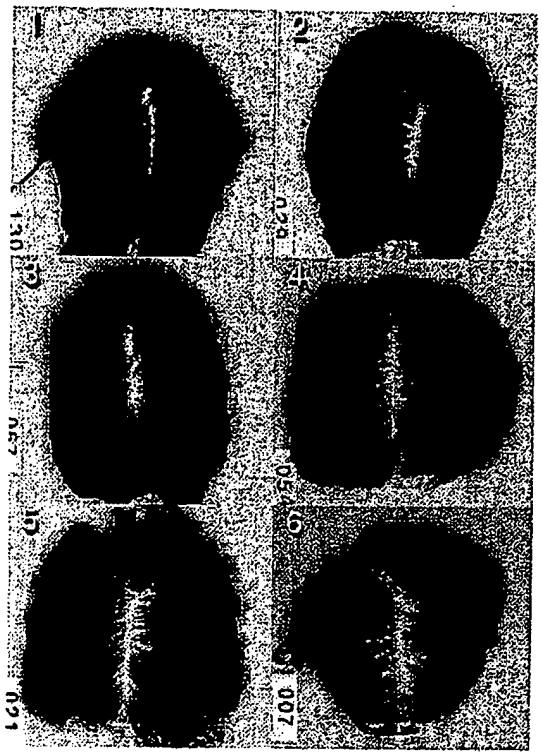

Fig. 1 Standard photographs of hair loss severity in Japanese women. Standard photographs constitute a 6-point scale from stage 1 (no hair loss) to stage 6 (detectable hair loss).

anagen hair was defined as a hair with a growth rate of 0.2 mm/day or more, and thick hair and thin hair were defined as over 80 μm in diameter and not over 40 μm in diameter [11], respectively.

2.4. Follicular units

Hair orifices were given *X*–*Y* coordinates on the Day 2 phototrichogram. Average-linkage hierarchical clustering, the so-called group average [12] was applied using a cluster program, the 'hclust' function of the S-PLUS2000 software (Mathematical Systems Inc., Tokyo, Japan). The conjugate line in a tree-view showed an assembly set with the nearest coordinates of the orifices. The connecting points of lines represented the distances between the orifices or the weighted mean between adjacent orifices. The calculated results were shown as tree-views. Hairs within a distance of 0.3 mm were tentatively considered to be a follicular unit. The following parameters were calculated using the phototrichogram data: density of follicular units (cm^{-2}), and ratio of each follicular unit to the total number of follicular units (%).

2.5. Statistical analysis

Analysis between groups of subjects was performed using Student's *t*-test. In all cases, tests with *p*-values of <0.05 were considered to be statistically significant.

3. Results

3.1. Japanese women with hair loss

We recruited 159 volunteer Japanese women aged between 14 years and 68 years. Severity of hair loss was determined as the mean of four investigator ratings compared with standard photographs on a 6-point scale from stage 1 (no hair loss) to stage 6 (detectable hair loss), as shown in Fig. 1. Hair loss in Japanese women is commonly characterized by a diffuse central pattern (Fig. 1). One hundred and thirteen subjects with a hair loss severity of 2 or less (71%) were classified as no or little hair loss subjects (Table 1). Forty-six subjects with a hair loss severity of over 2 were classified as hair loss subjects. Hair loss appeared in subjects aged over 38 years, and increased with age (Table 1).

3.2. Characteristic features of hair with aging in Japanese women

To characterize the effects of aging on hair properties, subjects aged between 14 years and 68 years with no or little hair loss were classified into 6 age groups as shown in Table 1 (upper parts). Hair densities were widely distributed in a range between 109 cm^{-2} and 417 cm^{-2}. Maximum mean hair densities were seen in subjects in their 20s. The mean hair density in those in their 20s was significantly higher than that in younger subjects (10s: $p = 0.0024$) and in older subjects (40s: $p = 0.0001$; 50s: $p < 0.0001$; and 60s: $p = 0.0017$) (Fig. 2).

Anagen hair ratios were distributed in a range between 58% and 98% (Table 1). Maximum mean anagen hair ratios were seen in subjects in their 10s. Mean hair anagen ratio declined with age, and the ratio in the 10s was significantly higher than that in the 40s ($p = 0.0230$) and 50s ($p = 0.0474$) (Table 1). Mean growth rates of anagen hair were distributed in a range between 279 μm and 565 μm (Table 1). Maximum mean growth rates of anagen hair were seen in subjects in their 20s. The mean growth rate of anagen hair declined with age, and was significantly higher in the 20s than in the 40s ($p = 0.0101$) and 50s ($p = 0.0155$) (Table 1).

Mean hair diameters were distributed in a range between 55 μm and 116 μm (Table 1). Maximum

Table 1. Characterization of subjects and their scalp hairs with aging

Age group	Sex	Age (years)	Rating of hair loss	Hair density (cm^{-2})	Anagen hair ratio (%)	Mean growth rate of anagen hair (μm/day)	Mean hair diameter (μm)	Thick hair ratio[a] (%)	Thin hair ratio[b] (%)	Follicular unit density (cm^{-2})
No or little hair loss										
10s	21	16.8 ± (11–19)	1.3 (1.0–2.0)	240.3 ± 37.9 (167–307)	89.2 ± 6.0 (76–97)	414 ± 42 (353–532)	79.3 ± 8.3 (61–92)	64.3 ± 13.7 (31–80)	12.9 ± 6.2 (3–25)	89.0 ± 9.0* (69–106)
20s	21	24.3 ± (20–29)	1.2 (1.0–1.9)	287.1 ± 54.3 (195–417)	87.4 ± 4.8 (79–96)	419 ± 53 (347–565)	80.7 ± 8.8 (64–99)	64.3 ± 14.3 (29–88)	12.9 ± 6.1 (3–30)	95.5 ± 9.7 (81–113)
30s	21	34.7 ± (30–39)	1.0 (1.0–2.0)	282.0 ± 44.4 (227–378)	86.0 ± 4.6 (75–94)	414 ± 37 (347–494)	85.9 ± 10.5 (70–116)	72.6 ± 10.6 (49–91)	11.4 ± 4.1 (3–20)	96.7 ± 9.9 (77–126)
40s	20	46.1 ± (40–49)	1.4 (1.0–2.0)	220.8 ± 41.8 (159–303)	84.5 ± 6.8 (72–95)	379 ± 41 (279–440)	87.1 ± 10.3 (69–109)	75.5 ± 9.6 (54–86)	12.7 ± 7.6 (0–30)	91.2 ± 15.3 (73–137)
50s	20	54.0 ± (50–59)	1.4 (1.0–2.0)	205.5 ± 50.5 (109–290)	84.1 ± 9.5 (65–96)	380 ± 45 (305–454)	85.0 ± 11.6 (55–112)	71.7 ± 19.1 (19–97)	11.9 ± 9.2 (0–41)	86.0 ± 15.4* (65–126)
60s	10	64.5 ± (60–68)	1.5 (1.0–2.0)	219.4 ± 42.4 (163–285)	86.2 ± 12.2 (56–98)	388 ± 50 (300–463)	81.1 ± 8.1 (69–91)	70.0 ± 14.3 (44–86)	7.6 ± 4.3* (3–16)	87.0 ± 18.3* (73–97)
Hair loss										
40s	10	41.6 ± (38–49)	2.8 (2.1–4.1)	188.7 ± 26.1 (148–237)	84.1 ± 5.4 (77–92)	363 ± 46 (283–426)	81.2 ± 11.1 (59–99)	70.8 ± 15.1 (42–88)	16.5 ± 12.2 (5–45)	86.5 ± 12.9 (71–109)
50s	14	54.4 ± (50–59)	2.8 (2.1–3.6)	178.8 ± 25.1 (145–232)	86.7 ± 5.9 (74–94)	399 ± 38 (339–456)	85.4 ± 19.6 (68–106)	75.2 ± 13.3 (42–88)	9.2 ± 7.5 (0–26)	81.3 ± 17.2 (72–97)
60s	22	64.1 ± (60–68)	2.3 (2.1–3.0)	179.9 ± 43.3 (95–251)	79.6 ± 10.0 (60–97)	317 ± 38 (256–416)	70.4 ± 11.0 (56–91)	49.4 ± 21.0 (15–84)	17.2 ± 10.5 (5–43)	90.9 ± 15.6 (56–113)

Data indicated mean ± S.D. The range of data showed in parentheses. [a]Thick hair ratio indicated percent of hairs of over 80 μm diameter. [b]Thin hair ratio indicated percent of hairs of not over 40 μm diameter. In subject groups with no or little hair loss, maximal value in each column were underlined. Significant differences vs. the underlined subject group were shown. In hair loss groups, significant difference vs. the same generation subject in no or little hair loss group were shown. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

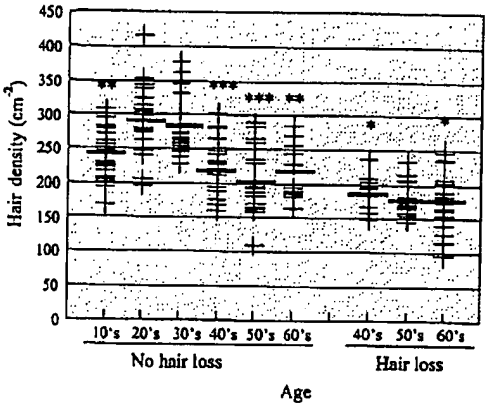

Fig. 2 Hair densities of subjects with or without hair loss. Hair densities and mean densities are indicated by crosses and bold bars, respectively. Asterisks indicate significant differences vs. hair densities of 20s group in a part of no or little hair loss subjects. Significant differences between those of same age group with and without hair loss are indicated in a part of hair loss subjects. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

mean diameters were seen in subjects in their 40s. Mean diameter increased until the 40s, then declined with age (Fig. 3), and was significantly higher in the 40s than in the 10s ($p = 0.0110$) and 20s ($p = 0.0414$) (Table 1). The ratio of thick hairs (over 80 μm in diameter) was coupled with mean hair diameter and ranged between 19% and 97% (Table 1). Maximum mean thick hair ratios were seen in subjects in their 40s. Mean thick hair ratio

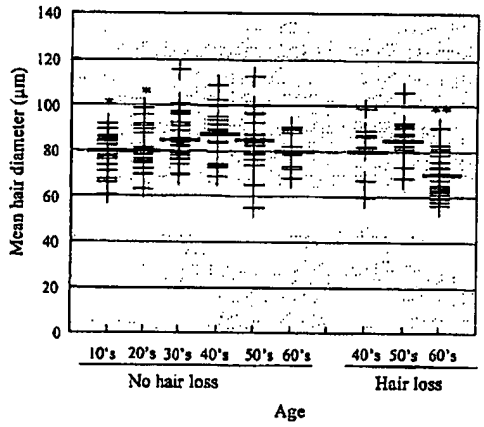

Fig. 3 Mean hair diameters of subjects with or without hair loss. Mean hair diameters and their means are indicated by crosses and bold bars, respectively. Asterisks indicate significant differences vs. mean hair diameters of 40s group in a part of no or little hair loss subjects. Significant differences between those of same age group with and without hair loss are shown in a part of hair loss subjects. $^{*}p < 0.05$; $^{**}p < 0.01$.

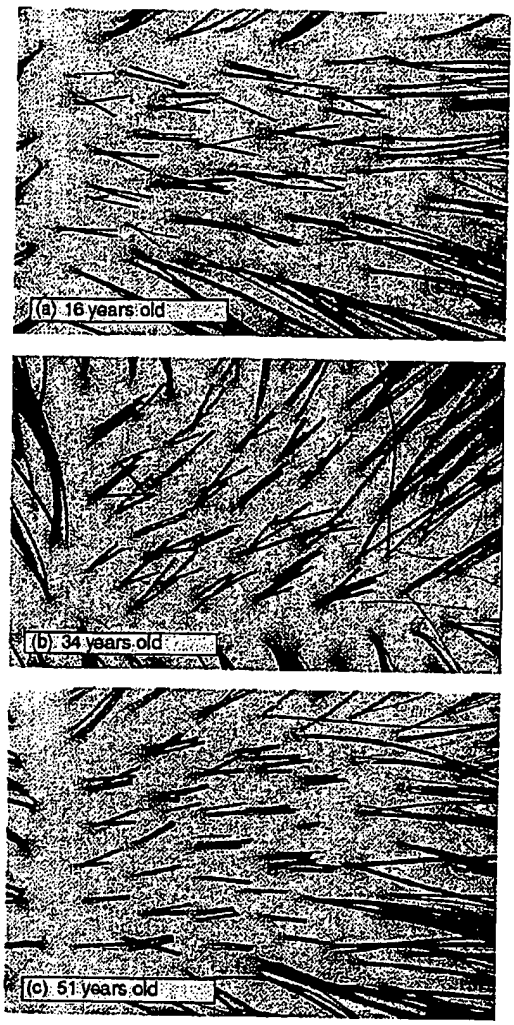

Fig. 4 Scalp images in women without hair loss. Digital image of cut area on vertex on Day 2 is shown. (a) 16 years old. Hair loss rating, 1.0; hair density, 203 cm^{-2}; anagen hair ratio, 95.1%; mean growth rate of anagen hair, 434 μm/day; mean hair diameter, 79.5 μm; thick hair ratio, 62.7%; thin hair ratio, 8.5%; follicular unit density, 77.5 cm^{-2}; ratios of one-, two-, three-, and four- and over-haired follicular units, 4.2, 50.0, 33.3, and 12.5, respectively. (b) 34 years old. Hair loss rating, 1.0; hair density, 350 cm^{-2}; anagen hair ratio, 86.6%; mean growth rate of anagen hair, 404 μm/day; mean hair diameter, 88.7 μm; thick hair ratio, 79.7%; thin hair ratio, 11.4%; follicular unit density, 96.0 cm^{-2}; ratios of one-, two-, three-, and four- and over-haired follicular units, 3.2, 45.2, 38.7, and 12.9, respectively. (c) 51 years old. Hair loss rating, 1.0; hair density, 239 cm^{-2}; anagen hair ratio, 86.6%; mean growth rate of anagen hair, 341 μm/day; mean hair diameter, 85.5 μm; thick hair ratio, 74.6%; thin hair ratio, 17.2%; follicular unit density, 79.4 cm^{-2}; ratios of one-, two-, three-, and four- and over-haired follicular units, 3.8, 50.0, 34.6, and 11.5, respectively.

in the 40s was higher than that in the 10s ($p = 0.0045$) and 20s ($p = 0.0046$) (Table 1). In contrast, the ratio of thin hairs (not over 40 μm in diameter) was much higher in younger women. Thin hair ratios were distributed in a range between 0% and 41% (Table 1). Maximum mean thin hair ratios were seen in subjects in their 20s. Mean thin hair ratio in the 20s was higher than that in the 60s ($p = 0.0181$) (Table 1).

Microscope images of subjects without hair loss are shown in Fig. 4. There were no marked differences in hair diameter or hair length in subjects of various ages (16, 34, and 51 years). Thus, hair density, follicular unit density, anagen hair ratio, and growth rate decreased with age from between the 20s and the 40s in Japanese women with no or little hair loss. Differences in hair density were particularly noticeable. Younger women in their 10s and 20s tended to have smaller hair diameters.

3.3. Characteristic features of hair in Japanese women with hair loss

Microscope images of subjects with hair loss were shown in Fig. 5. In a subject aged 51 years with a hair loss rating of 3.6 (Fig. 5a), hair diameter and hair growth were little different from those of women without hair loss (Fig. 4c). However, in a subject aged 60 years with a hair loss rating of 5.3, a number of thin hairs, hairs with slower growth, and extremely low hair density were observed compared with no hair loss subjects (Fig. 5b).

To clarify the features of hair with hair loss, 46 subjects with a hair loss rating of over 2 were reclassified by age (Table 1, lower part). According to a cohort analysis, mean hair densities were reduced compared with those in the no or little hair loss group (Table 1, Fig. 2; 40s: $p = 0.0354$; 50s: $p = 0.0782$; 60s: $p = 0.0224$). The reduction rates were 14.5%, 13.0% and 18.0% in subjects in their 40s, 50s, and 60s, respectively. Furthermore, compared with younger women in their 20s, the reduction was most notable (37.3%) in subjects in their 60s. Cohort analysis also indicated significantly reduced hair growth rate ($p = 0.0036$), mean hair diameter ($p = 0.0055$), and thick hair ratio ($p = 0.0085$), and increased thin hair ratio ($p = 0.0096$), in subjects in their 60s with hair loss (Table 1). These results might be caused by the higher hair loss rating in subjects in their 60s (mean hair loss rating: 4.3) than in subjects in their 40s or 50s (mean rating in 40s and 50s subjects: 2.8).

To investigate the relationship between hair loss and hair characteristics, subjects aged 40 years and over, and a subject aged 38 years with hair loss, were reclassified by severity of hair loss (Table 2).

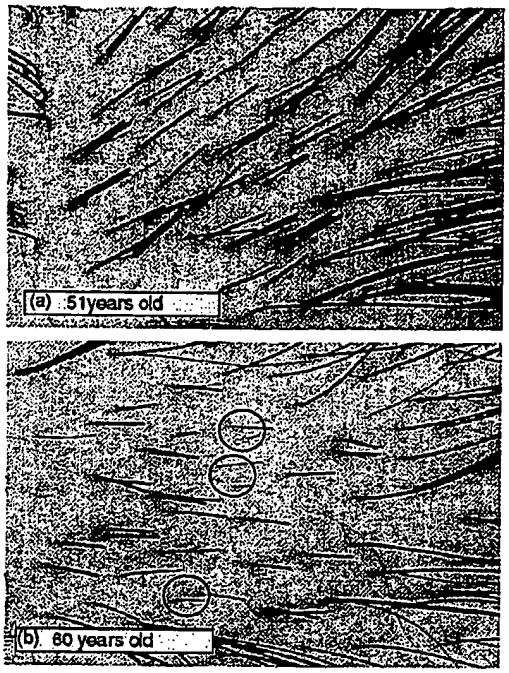

Fig. 5 Scalp images in women with hair loss. Digital image of cut area on vertex at Day 2 is shown. (a) 51 years old. Hair loss rating, 3.6; hair density, 153 cm^{-2}; anagen hair ratio, 94.3%; mean growth rate of anagen hair, 373 μm/day; mean hair diameter, 72.9 μm; thick hair ratio, 65.4%; thin hair ratio, 21.2%; follicular unit density, 82.0 cm^{-2}; ratios of one-, two-, three-, and four- and over-haired follicular units, 37.9, 44.8, 13.8, and 3.4, respectively. (b) 60 years old. Hair loss rating, 5.3, hair density, 116 cm^{-2}; anagen hair ratio, 81.0%; mean growth rate of anagen hair, 256 μm/day; mean hair diameter, 59.7 μm; thick hair ratio, 30.6%; thin hair ratio, 30.6%; follicular unit density, 63.1 cm^{-2}; ratios of one-, two-, three-, and four- and over-haired follicular units, 50.0, 38.5, 11.5, and 0, respectively. Note few vellus-like hairs and slower growth of hairs in (a). Unclear hairs in grouping into follicular units are indicated in circles in (b).

The mean age of subjects significantly increased with progressing hair loss compared with subjects without hair loss (hair loss rating 3–4: $p = 0.0059$; hair loss rating > 4: $p < 0.0001$). Mean hair densities were significantly reduced with progressing hair loss (hair loss rating 2–3: $p = 0.0205$; hair loss rating > 4: $p = 0.0001$). Mean anagen hair ratio and mean growth rate of anagen hair were reduced in severe hair loss subjects with a hair loss rating of over 4 (mean anagen hair ratio: $p = 0.0338$; mean growth rate of anagen hair: $p = 0.0002$). However, anagen hair ratios were widely distributed without relation to hair loss severity. Mean hair diameters were reduced in conjunction with mean thick hair ratio, with progressing severity of hair loss (mean

Table 2. Characterization of hairs in subjects with hair loss

Severity of hair loss	n	Age (years)	Hair density (cm^{-2})	Anagen hair ratio (%)	Mean growth rate of anagen (μm)	Mean hair diameter (μm)	Thick hair ratio[b] (%)	Thin hair ratio[c] (%)
1	50	52.5 ± 6.7	214.4 ± 45.3	84.7 ± 9.0	381 ± 44	85.1 ± 10.9	72.9 ± 14.8	11.4 ± 7.9
		(40–68)	(109–303)	(58–98)	(279–463)	(55–112)	(19–97)	(0–41)
2–3	22	53.1 ± 7.5	189.7 ± 27.1	85.9 ± 5.9	380 ± 48	82.8 ± 11.4	71.5 ± 19.2	11.5 ± 10.6
		(38–68)	(145–237)	(74–97)	(283–456)	(59–106)	(15–88)	(0–45)
3–4	11	59.1 ± 7.7	193.2 ± 37.1	81.3 ± 10.3	363 ± 38	75.0 ± 9.9**	55.8 ± 18.6**	15.6 ± 7.9
		(47–68)	(148–251)	(60–94)	(306–426)	(59–92)	(24–80)	(5–29)
>4	13	62.2 ± 7.4	157.6 ± 35.2**	78.6 ± 9.1	329 ± 38**	69.9 ± 10.7**	48.1 ± 19.3**	19.0 ± 11.2**
		(46–68)	(95–234)	(62–95)	(256–389)	(56–89)	(19–77)	(6–43)

Data indicated mean ± S.D. The range of data showed in parenthesis. [a] Rating of hair loss. [b] Thick hair ratio indicated percent of hairs over 80 μm diameter. [c] Thin hair ratio indicated percent of hairs over 40 μm diameter. Significant differences vs. subject group with ≤2 of rating were shown; $^{*}P < 0.05$, $^{**}P < 0.001$.

hair density: hair loss rating 3–4: $p = 0.0067$, hair loss rating > 4: $p < 0.0001$; mean thick hair ratio: hair loss rating 3–4: $p = 0.0087$, hair loss rating > 4: $p < 0.0001$). Mean thin hair ratios increased with progressing hair loss (hair loss rating > 4: $p = 0.0059$), but the difference was considerably smaller than the reduction of mean thick hair ratio. Thus, in Japanese women with hair loss, there were reductions in hair density in the vertex area, anagen hair ratio, hair growth rate, thick hair ratio, and mean hair diameter, and an increase in thin hair ratio, with progressing hair loss. In particular, prominent changes were observed in hair density (26% reduction in subjects with a hair loss rating of >4) and thick hair ratio (34% reduction hair loss rating >4).

3.4. Follicular units

It is well known that scalp hairs grow in groups mainly consisting of three hairs, the so-called 'trio arrangement' [13]. Hair groups defined as follicular units had a well-circumscribed structure comprising two- to four-terminal hair follicles, and one or, rarely, two vellus follicles [14]. To clarify the relationship between follicular units and hair density with progressing hair loss, we analyzed the numbers of follicular units and of hairs within follicular units. Follicular units were relatively easily recognized in scalps without hair loss (Fig. 4), but not in scalps with hair loss. For example, there were relatively large distances between hair orifices, as shown in the circles in Fig. 5b. It was difficult to assess whether hairs were grouped into one-haired follicular units or two-haired follicular units. Although follicular units should be determined by the examination of biopsy specimens, this invasive method was ethically unacceptable to a number of the subjects. To overcome this problem, we developed a non-invasive method using computer analysis of scalp imaging. An average-linkage hierarchical clustering was obtained using a cluster program and was displayed as a tree-view (Fig. 6). The connecting points of lines represented the distance in terms of X–Y coordinates between hair orifices or their weighted mean points, as shown on the vertical axis. A tree-view in a subject without hair loss shows a relatively regular pattern (Fig. 6a). In contrast, a subject with hair shows an irregular pattern containing connecting points at various heights (Fig. 6b). We tentatively defined a follicular unit to consist of hairs within 0.3 mm, according to data from several biopsy specimens from Japanese women (AI, unpublished data) and the literature [15].

Follicular unit densities were distributed in a range between 65 cm^{-2} and 137 cm^{-2} in subjects

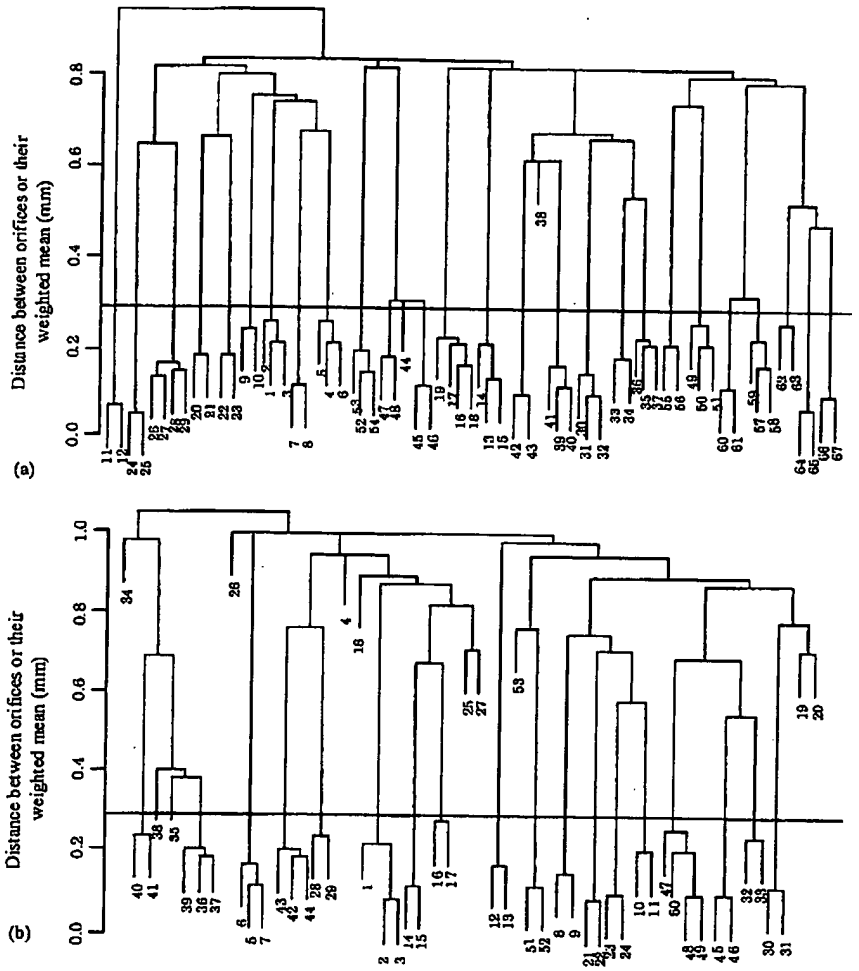

Fig. 6 Clustering trees for follicular units. Hair follicles orifices were given X–Y coordinates on the scalp image. Average-linkage hierarchical clustering was applied using a cluster program, the 'hclust' function of the S-PLUS2000 software. Calculated results are shown as tree-views. (a) A woman without hair loss, 51 years old, shown in Fig. 4c and (b) a woman with hair loss, 51 years old, shown in Fig. 5a. Numbers indicate individual orifices of hair follicles. Conjugate lines in tree-views indicate an assembly set with a nearest coordinate of orifices. Branching points of lines represent the distance between orifices or the weighted means between adjacent orifices. Orifices within 0.3 mm (a longest horizontal line) are tentatively put together in a follicular unit. Two one-haired follicular units (7.1%), indicated by Nos. 38 and 44, 15 two-haired follicular units (53.6%), 9 three-haired follicular units (32.1%), and 2 four-haired follicular units (7.1%) were found in tree-view (a). Eleven one-haired follicular units (37.9%), indicated by Nos. 4, 18, 19, 20, 25, 26, 27, 34, 35, 38, and 53, 13 two-haired follicular units (44.8%), 4 three-haired follicular units (13.8%), and 1 four-haired follicular unit (3.4%) were found in tree-view (b). Note the irregular pattern and more one-haired follicular units in tree-view (b).

with no or little hair loss (Table 1). Maximum mean follicular unit densities were seen in subjects in their 30s. Mean follicular unit density in the 30s was higher than that in the 10s ($p = 0.0122$), the 50s ($p = 0.0114$), and the 60s ($p = 0.0119$) (Table 1). In subjects with hair loss, there were no significant differences in the density of follicular units; however, the ratio of one-haired follicular units increased significantly with progressing hair loss (hair loss rating 2–3: $p = 0.0015$; hair loss rating 3–4: $p = 0.0102$; hair loss rating >4: $p = 0.001$) (Table 3). In addition, the ratios of three-haired follicular units (hair loss rating 2–3: $p = 0.0007$; hair loss rating >4: $p = 0.0096$) and four- or more-haired follicular units (hair loss rating 2–3: $p = 0.0468$; hair loss rating >4: $p = 0.0016$), but not two-haired follicular units, declined with progressing hair loss (Table 3). These data show that reduced hair density in Japanese women with hair loss correlated with increased numbers of one-haired follicular units,

Table 3 Follicular units in subjects with hair loss

Severity of hair loss	Density of follicular unit (cm^{-2})	Ratio of follicular unit (%)			
		One-haired	Two-haired	Three-haired	Four- or more-haired
1–2	288.3 ± 54.2 (165–377)	24.9 ± 14.3 (0–68)	42.9 ± 14.3 (21–75)	23.4 ± 11.7 (0–50)	8.8 ± 8.7 (0–39)
2–3	288.7 ± 70.7 (77–412)	36.8 ± 13.7** (7–71)	44.9 ± 11.5 (25–69)	13.6 ± 8.4** (0–36)	4.7 ± 6.2* (0–22)
3–4	187.3 ± 75.1 (77–413)	36.7 ± 17.5 (20–45)	38.8 ± 8.3 (25–52)	20.1 ± 8.6 (7–35)	4.4 ± 2.3 (0–8)
4–5	83.8 ± 46.5 (10–161)	43.8 ± 13.7** (19–61)	41.1 ± 10.6 (26–62)	14.3 ± 7.0** (3–32)	0.8 ± 2.1** (0–7)

Data indicated mean ± S.D. The range of data showed in parenthesis. Rating of hair loss. Significant differences vs. subject group with ≤2 of rating were shown; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

and with reduced three and more-haired follicular units.

4. Discussion

It is well known that scalp hair density in adults decreases gradually with age and shows little difference between the sexes [16]. We demonstrated that hair density and follicular unit density declined with aging in Japanese women without the appearance of hair loss. Hair densities were widely distributed in a range from 109 cm^{-2} to 417 cm^{-2} in women aged under 50s, and the mean hair density in the 20s was 287.1 ± 54.3 (S.D.) cm^{-2}. It has been reported that, in Caucasian women, mean hair densities were 300 ± 20 (S.D.) cm^{-2} in 20 women aged 30 years or under without hair loss [17], and 293 ± 61.3 (S.E.M.) cm^{-2} at age 35 years [6]. We also observed that mean hair density in subjects in their 60s without hair loss was 219.4 ± 42.4 (S.D.) cm^{-2}. In Caucasian women at age 70 years, mean hair density was 211 ± 55.1 (S.E.M.) cm^{-2}. These data show that there are few racial differences in hair density in females with aging, but there are extreme inter-individual differences.

We demonstrated that the mean anagen ratio and the mean growth rate of anagen hairs declined with progressing age. It was reported that, in Caucasian women without hair loss, hair growth rate in seven younger women (average age 19 years) was 0.427 mm/day, compared with 0.401 mm/day in five mature women (average age 42.8 years) [18]. In Japanese females, growth rates were slightly lower, at 0.419 mm/day in the 10s and 0.379 mm/day in the 40s, compared with the Caucasian data, but similarly declined with aging.

It has been reported that, in Caucasian women without hair loss, mean hair diameters of the major and minor axes were 74.1 ± 11.3 (S.D.) μm and 52.3 ± 6.6 (S.D.) μm, respectively [6]. We observed a larger mean hair diameter, 87.1 ± 10.3 (S.D.) μm, in the major axis in Japanese subjects in their 40s without hair loss. However, the thin hair ratio in Japanese women (12.9 ± 6.1 (S.D.) % in subjects in their 20s) was higher than that in Caucasians (2.29–3.87% in the major axis) [6]. We also observed a relatively lower mean hair density and mean follicular unit density in women in their 10s, and a relatively lower mean hair diameter and mean hair thick ratio in women in their 10s and 20s. Since the hairs in younger women are in the maturation process, significantly lower data for hair density and diameter might be obtained. However, geo-environmental changes and changes in living conditions may generate differences in hair properties between

younger and older generations. Further studies are required to explain the features of hair in younger subjects.

Severe female hair loss such as Ludwig pattern Type III [1], and the Women's Alopecia Severity Scale stages 4 and 5 [19], which involve diffuse hair loss on most or all of the scalp, appear to be rare in Japanese women [9]. Therefore, to classify Japanese women with light to moderate hair loss, we developed a method using six standard photographs. According to a cohort analysis, the prevalence of hair loss subjects in their 60s was markedly higher than that in subjects in their 40s and 50s (Table 1); consistent with a study of Caucasian females [6].

FPHL is generally thought to be the female equivalent of male balding [20]. FPHL includes heterogeneous hair disorders and can show one of three patterns: frontal accentuation ('Christmas tree pattern'), diffuse central, or vertex/frontal (androgenetic alopecia) [2]. In Caucasian females, female androgenetic alopecia is most common (77%) followed by chronic telogen effluvium (21%) [21]. By contrast, we observed that the hair loss pattern in Japanese women was commonly classified as diffuse central, not female androgenetic alopecia. The major characteristic features of hair loss in Japanese women were reduced hair density and thick hair ratio, which were consistent with previous reports [9]. Therefore, common hair loss in Japanese women should be classified as FPHL, especially chronic telogen effluvium, with diagnostic characteristics of increasing hair loss from the centre towards the front of the scalp, and a decrease in hair density. There were significant differences in thin hair ratio and anagen hair ratio between subjects with and without hair loss, but the differences were relatively small. Most thin hairs were long in length, similar to terminal hairs, and thus may have been intrinsic thin hairs, not from any miniaturization of hair follicles as with androgenetic alopecia [22]. Therefore, miniaturization of hair follicles might be rare in FPHL in Japanese women.

Numbers of follicular units and the number of hairs in follicular units were strongly related to hair density. The follicular unit is a well-circumscribed structure comprising one- to four-terminal hair follicles, and the hairs in a follicular unit have associated sebaceous lobules and a shared arrector pili muscle [14,15,23]. For ethical reasons, we were unable to determine follicular units using biopsy specimens. We therefore developed a non-invasive, objective method using tree-view analysis of magnified scalp images. In our analysis, a complex arrangement of hairs on imaging was easily converted to a clear, graphic tree-view (Fig. 6). This non-invasive method might be inferior in accuracy to the invasive biopsy method, but was better in terms of the effectiveness of data analysis and was more acceptable to the subjects. We estimated that the analytical data were sufficiently accurate to detect the characteristics of follicular units compared with visual imaging data. Thus, mean follicular unit density decreased with progressing age after peaking in the 30s for subjects with no or little hair loss (Table 1). In subjects with hair loss, the mean ratio of one-haired follicular units significantly increased with progressing hair loss, and the mean ratios of three- and four- and more-haired follicular units were reduced with progressing hair loss. The evidences above may indicate increased empty follicles or miniaturized follicles, as has been described [9].

Taken together, we observed thick and dense scalp hairs in Japanese women aged 20–40 years. However, subsequently, hair density, diameter, anagen ratio and the growth rate of anagen hairs gradually decreased with age. From approximately 40 years of age, FPHL was seen to begin occurring. We conclude that major causes of FPHL are reduced hair density due to reductions in three and more-haired follicular units, and increased one-haired follicular units, and a reduced thick hair ratio, but not an increase in vellus-like hairs.

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